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(54) Title: NOVEL RECEPTOR LIGANDS AND GENETIC SEQUENCES ENCODING SAME

(57) Abstract

The present invention relates generally to novel receptor ligands and to genetic sequences encoding same. The novel receptor ligands and their encoding genetic sequences are useful in the development of a wide range of agonist, antagonist, therapeutic and diagnostic reagents based on ligand-receptor interaction. The present invention further provides a means for identifying ligands without knowledge of their receptor and a means for identifying receptors.

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NOVEL RECEPTOR LIGANDS AND GENETIC SEQUENCES ENCODING SAME

The present invention relates generally to novel receptor ligands and to genetic sequences encoding same. The novel receptor ligands and their encoding genetic sequences are useful in the development of a wide range of agonist, antagonist, therapeutic and diagnostic reagents based on ligand-receptor interaction. The present invention further provides a means for identifying ligands without knowledge of their receptor and a means for identifying receptors.

Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined at the end of the description.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The rapidly increasing sophistication of recombinant DNA technology is greatly facilitating research into the medical and allied health fields. Research into cellular growth factors such as cytokines is of particular importance due to their involvement in proliferation, differentiation and function of a wide variety of cells. Administration of recombinant growth factors or of molecules capable of regulating growth factor function and/or synthesis is becoming increasingly the focus of medical research into the treatment of a range of conditions.

Many cellular growth factors and other extracellular mediators of cell function exert their actions by interacting with cell surface receptor protein tyrosine kinases (RPTKs). Ligand binding to such receptors usually results in receptor aggregation, cross-phosphorylation on tyrosine residues of the receptor cytoplasmic domains, activation of the intrinsic kinase activity of the cytoplasmic domains and phosphorylation of down-stream cell signalling molecules

(Schlessinger and Ullrich *Neuron 9:* 383-391, 1990; Flanti *et al Ann. Rev. Biochem. 61:* 453-481, 1993; van der Greer and Hunter *Ann. Rev. Cell. Biol. 10:* 251-337, 1994).

RPTKs can be subdivided into several classes dependent primarily on the predicted structural features of their extracellular domains and whether or not they contain an uninterrupted tyrosine kinase domain in the cytoplasmic portion of the receptor (Hilton In "Guidebook to Cytokines and Their Receptors" N.A. Nicola ed, Oxford University Press, Oxford pp 8-16, 1994). The largest class of RPTKs is known as the eph-related kinases (ERKs) and include the tyrosine kinases eph, elk, cck, eek, erk, cek 4-10, mek 4, hek, tk2, tyro 5 and sek. They are characterised by an extracellular sequence that includes a single cysteine-rich repeat domain followed by two fibronectin type III domains and an uninterrupted tyrosine kinase domain in the cytoplasmic region.

Despite the fact that ERKs comprise the largest subclass of RPTKs, most of the ERKs were identified without prior knowledge of their biological function nor of the ligands with which they interact. Nevertheless, the expression patterns of the ERKs suggest that they might have important roles in early vertebrate development and in brain function and hemopoietic cell function as well as functions on other organs in the adult (Cheng and Flanagan *Cell 79:* 157-168, 1994; Shao *et al J. Biol. Chem. 270:* 3467-3470, 1995; Beckman *et al EMBO J. 13:* 3757-3762, 1994).

There is clearly a need, therefore, to identify and characterise ERKs. There is also an important need to identify ligands for ERKs with or without prior knowledge of the specific ERK to which a ligand might interact.

Recently, some ligands that can bind to ERKs have been identified and are referred to as "ligands for ERKs" (LERKs) amongst other names. The first of these, LERK1, is equivalent to the B61 gene product previously identified as an interleukin-1 - or tumour necrosis factor-inducible product of human umbilical vein endothelial cells (Holzman *et al Mol. Biol. 10:* 5830-5838, 1990; Beckmann *et al EMBO J. 13:* 3757-3762, 1994). LERK2 appears to be

identical to human ELK ligand (ELK-L) and the human homologue of the mouse proteins elg 2 (eph-ligand 2) and cek5 L (cek5-ligand) (Beckmann *et al EMBO J. 13:* 3757-3762, 1994; Fletcher *et al Genomics 24:* 127-132, 1994; Shao *et al J. Biol. Chem. 269:* 26602-26609, 1994; Davis *et al Science 266:* 816-819, 1994). LERK3 is nearly identical to EHK1-L (ehk1-ligand) Kozlosky *et al Oncogene 10:* 299-306, 1995; Davis *et al Science 266:* 816-819, 1994), LERK4 is unique (Kozlosky *et al Oncogene 10:* 299-306, 1995) and the cek7 ligand is identical to elf-1 (eph ligand family-1) (Shao *et al J. Biol. Chem. 270:* 3467-3470, 1995); Cheng and Flanagan *Cell 79:* 157-168, 1994). All of these ligands appear to be cell surface-associated either through a C-terminal glycosyl-phosphatidyl inositol (GPI)-linkage (LERKs 1, 3 and 4) or through a traditional transmembrane domain (LERK2). Most appear to require cell surface expression or soluble dimer forms in order to activate the tyrosine kinase activity of the appropriate ERKs (Davis *et al Science 266:* 816-819, 1994). Moreover, none of the LERKs described so far show an absolute specificity for one ERK. For example, LERK1 binds to hek, elk and eck; LERKs 2, 3 and 4 bind to hek and elk and cek 7-L binds to sek and mek 4 with equilibrium dissociation constants varying from 1 nM to 500 nM.

In accordance with the present invention, the inventors have identified a new LERK which will assist in the development of a range of new therapeutics and diagnostics and in the identification of new ERKs.

Accordingly, one aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a LERK or part thereof, said nucleotide sequence or its complementary form encoding at least one of the following amino acid sequences:

(i) VXWXSXN [SEQ ID NO:1];

(ii) DXXDIXCP [SEQ ID NO:2];

(iii) EXYXLYXVXXXXXXXC [SEQ ID NO:3]; and/or

(iv) KFQXXXXXXXGXEFXXXHXYYXI [SEQ ID NO:4];

wherein X is any amino acid residue.

Preferably, the nucleotide sequence or its complementary form encodes a product comprising at least two of, more preferably at least three of and even more preferably all four of amino acid sequences SEQ ID NO:1 to SEQ ID NO:4.

In this regard, the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a LERK or part thereof, said nucleotide sequence or its complementary form encoding an amino acid sequence selected from the list consisting of:

(i) VXWXSXN [SEQ ID NO:1];

(ii) DXXDIXCP [SEQ ID NO:2];

(iii) EXYXLYXVXXXXXXXC [SEQ ID NO:3]; and

(iv) KFQXXXXXXXGXEFXXXHXYYXI [SEQ ID NO:4];

wherein X is any amino acid residue.

Preferably, the nucleic acid molecule of the present invention encodes a LERK referred to herein as "NLERK2", said nucleic acid molecule selected from the list consisting of:

(i) a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:5;

(ii) a nucleic acid molecule comprising a sequence of nucleotides having at least about 70% similarity to the nucleotide sequence set forth in SEQ ID NO:5; and

(iii) a nucleic acid molecule capable of hybridising under low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:5.

The nucleotide molecule is preferably derivable from the human genome but genomes and nucleotide sequences from non-human animals are also encompassed by the present invention. Non-human animals contemplated by the present invention include livestock animals (e.g. sheep, cows, pigs, goats, horses, donkeys), laboratory test animals (e.g. mice, rats, guinea pigs, hamsters, rabbits), domestic companion animals (e.g. dogs, cats), birds (e.g. chickens, geese, ducks and other poultry birds, game birds, emus, ostriches) and captive wild or tamed animals

(e.g. foxes, kangaroos, dingoes).

Preferred human genomic sequences include sequences from brain, liver, kidney, neonatal, embryonic, cancer or tumour-derived tissues.

Once a novel nucleotide sequence is obtained as indicated above encoding a LERK, oligonucleotides may be designed which bind cDNA clones with high stringency. Direct colony hybridisation may be employed or PCR amplication may be used. The use of oligonucleotide primers which bind under conditions of high stringency ensures rapid cloning of a molecule encoding the novel LERK and less time is required in screening out cloning artefacts. However, depending on the primers used, low or medium stringency conditions may also be employed.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

Accordingly, another aspect of the present invention contemplates a method for cloning a nucleotide sequence encoding a novel LERK, said method comprising searching a nucleotide data base for a sequence which encodes at least one of amino acid sequences SEQ ID NO:1 to SEQ ID NO:4, designing one or more oligonucleotide primers based on a nucleotide sequence located in the search, screening a nucleic acid library with said one or more oligonucleotides and obtaining a clone therefrom which encodes said novel LERK or part thereof.

Preferably, the nucleic acid library is a cDNA, genomic or mRNA library.

Preferably, the nucleic acid library is a cDNA expression library.

Preferably, the nucleotide data base is of human origin such as from brain, liver, kidney, neonatal tissue, embryonic tissue, tumour or cancer tissue.

In one preferred embodiment, the oligonucleotide primers are selected from SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13.

In a particularly preferred embodiment the LERK is selected from the list consisting of:

(i) a nucleotide sequence substantially as set forth in SEQ ID NO:5;

(ii) a nucleotide sequence having at least about 75% similarity to the nucleotide sequence set forth in SEQ ID NO:5; and

(iii) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence as set forth in SEQ ID NO:5.

In an alternative aspect of this embodiment, medium stringency conditions are employed. In another alternative aspect, high stringency conditions are employed.

Another aspect of the invention is directed to the genomic gene and to 3′ and 5′ regions thereof. The 3′ region of the NLERK2 gene is represented in SEQ ID NO:14 and the 5′ region is shown in SEQ ID NO:15.

Accordingly, another aspect of the present invention is directed to a nucleic acid molecule corresponding to or derived from the 3′ region of the genomic gene encoding NLERK2 or its derivatives, said nucleic acid molecule or its complementary form having a nucleotide sequence substantially as set forth in SEQ ID NO:14 or having at least 75% similarity thereto or capable of hybridising thereto under low stringency conditions. Other levels of stringency such as

medium and high may also be used.

A further embodiment is directed to a nucleic acid molecule corresponding to or derived from the 5′ region of the genomic gene encoding NLERK2 or its derivatives, said nucleic acid molecule or its complementary form having a nucleotide sequence substantially as set forth in SEQ ID NO:15 or having at least 75% similarity thereto or capable of hybridising thereto under low stringency conditions. Other levels of stringency such as medium and high may also be used.

The present invention also extends to hybrid genetic sequences comprising a coding region of a structural gene flanked or fused to one or both of SEQ ID NO:14 and/or SEQ ID NO:15 or derivatives thereof.

Still another embodiment contemplates the promoter or a functional part thereof of the genomic gene encoding NLERK2. The promoter may readily be obtained by, for example, "chromosome walking".

Another aspect of the present invention is directed to NLERK2 including a recombinant polypeptide having an ability to interact with an ERK wherein said NLERK2 or said polypeptide is encoded by a nucleotide sequence translatable to at least one of amino acid sequences SEQ ID NO:1 to SEQ ID NO:4 and subsequently cloned by designing at least one oligonucleotide probe based on said nucleotide sequence and using same to clone a nucleotide sequence encoding said LERK or polypeptide from a DNA or RNA library.

This aspect of the present invention extends to recombinant NLERK2 or a polypeptide having NLERK2-like properties but does not extend to known LERKs such as LERK1, LERK2, LERK3 or LERK4.

In a particularly preferred embodiment, the present invention provides NLERK2 which comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 70% similarity, preferably at least about 80% similarity, more preferably at least about 90% similarity or most preferably at least about 95 to 100% similarity to all or part of the sequence set forth in SEQ ID NO:6. This aspect encompasses NLERK2 and derivatives thereof such as fragments, parts, portions, mutants and homologues.

In a related embodiment, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the amino acid sequence set forth in SEQ ID NO:6 or to an amino acid sequence having at least about 70% or above similarity to all or part of the amino acid sequence set forth in SEQ ID NO:6.

As stated above, the present invention further contemplates a range of derivatives of NLERK2. Derivatives include fragments, parts, portions, mutants, homologues and analogues of the NLERK2 polypeptide and corresponding genetic sequence. Derivatives also include single or multiple amino acid substitutions, deletions and/or additions to NLERK2 or single or multiple nucleotide substitutions, deletions and/or additions to the genetic sequence encoding NLERK2. "Additions" to amino acid sequences or nucleotide sequences include fusions with other peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to "NLERK2" includes reference to all derivatives thereof including functional derivatives or NLERK2 immunologically interactive derivatives.

Analogues of NLERK2 contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic

anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-

isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 1.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

The present invention further contemplates chemical analogues of NLERK2 capable of acting as antagonists or agonists of NLERK2 or which can act as functional analogues of NLERK2. Chemical analogues may not necessarily be derived from NLERK2 but may share certain conformational similarities. Alternatively, chemical analogues may be specifically designed to mimic certain physiochemical properties of NLERK2. Chemical analogues may be chemically synthesised or may be detected following, for example, natural product screening.

The identification of NLERK2 permits the generation of a range of therapeutic molecules capable of modulating expression of NLERK2 or modulating the activity of NLERK2. Modulators contemplated by the present invention includes agonists and antagonists of NLERK2 expression. Antagonists of NLERK2 expression include antisense molecules, ribozymes and co-suppression molecules. Agonists include molecules which increase promoter ability or interfere with negative regulatory mechanisms. Agonists of NLERK2 include molecules which overcome any negative regulatory mechanism. Antagonists of NLERK2 include antibodies and inhibitor peptide fragments.

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle

D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser

D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr

L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

These types of modifications may be important to stabilise NLERK2 if administered to an individual or for use as a diagnostic reagent.

Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

Another embodiment of the present invention contemplates a method for modulating expression of NLERK2 in a human, said method comprising contacting the NLERK2 gene encoding NLERK2 with an effective amount of a modulator of NLERK2 expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of NLERK2. For example, a nucleic acid molecule encoding NLERK2 or a derivative thereof may be introduced into a cell to enhance the ability of that cell to survive, conversely, NLERK2 antisense sequences such as oligonucleotides may be introduced to decrease the survival capacity of any cell expressing the endogenous NLERK2 gene.

Another aspect of the present invention contemplates a method of modulating activity of NLERK2 in a human, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease NLERK2 activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative of NLERK2 or its receptor or a chemical analogue or truncation mutant of NLERK2 or its receptor.

Accordingly, the present invention contemplates a pharmaceutical composition comprising NLERK2 or a derivative thereof or a modulator of NLERK2 expression or NLERK2 activity and one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to as the active ingredients.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion or may be in the form of a cream or other form suitable for topical application. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 ug and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 µg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 µg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating NLERK2 expression or NLERK2 activity. The vector may, for example, be a viral vector.

Still another aspect of the present invention is directed to antibodies to NLERK2 and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to NLERK2 or may be specifically raised to NLERK2 or derivatives thereof. In the case of the latter, NLERK2 or its derivatives may first need to be associated with a carrier molecule. The antibodies and/or recombinant NLERK2 or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents.

For example, NLERK2 and its derivatives can be used to screen for naturally occurring antibodies to NLERK2. These may occur, for example in some autoimmune diseases. Alternatively, specific antibodies can be used to screen for NLERK2. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of NLERK2 levels may be important for diagnosis of certain cancers or a predisposition to cancers or for monitoring certain therapeutic protocols.

Antibodies to NLERK2 of the present invention may be monoclonal or polyclonal. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a diagnostic tool for assessing apoptosis or monitoring the program of a therapeutic regimin.

For example, specific antibodies can be used to screen for NLERK2 proteins. The latter would be important, for example, as a means for screening for levels of NLERK2 in a cell extract or other biological fluid or purifying NLERK2 made by recombinant means from

culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of NLERK2.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of NLERK2, or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates a method for detecting NLERK2 in a biological sample from a subject said method comprising contacting said biological sample with an antibody specific for NLERK2 or its derivatives or homologues for a time and under conditions sufficient for an antibody-NLERK2 complex to form, and then detecting said complex.

The presence of NLERK2 may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These, of course, includes both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain NLERK2 including cell extract, tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

In the typical forward sandwich assay, a first antibody having specificity for the NLERK2 or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however,

a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescene and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

The present invention also contemplates genetic assays such as involving PCR analysis to detect NLERK2 gene or its derivatives. Alternative methods or methods used in conjunction include direct nucleotide sequencing or mutation scanning such as single stranded

conformation polymorphoms analysis (SSCP) as specific oligonucleotide hybridisation, as methods such as direct protein truncation tests.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli*, *Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and a mammalian and more particularly a human NLERK2 gene portion, which NLERK2 gene portion is capable of encoding an NLERK2 polypeptide or a functional or immunologically interactive derivative thereof.

Preferably, the NLERK2 gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said NLERK2 gene portion in an appropriate cell.

In addition, the NLERK2 gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

The present invention also extends to any or all derivatives of NLERK2 including mutants, part, fragments, portions, homologues and analogues or their encoding genetic sequence including single or multiple nucleotide or amino acid substitutions, additions and/or deletions to the naturally occurring nucleotide or amino acid sequence.

The NLERK2 and its genetic sequence of the present invention will be useful in the generation of a range of therapeutic and diagnostic reagents and will be especially useful in the detection of a corresponding ERK. For example, recombinant NLERK2 may be bound or fused to a reporter molecule capable of producing an identifiable signal, contacted with a cell or group of cells putatively carrying ERKs and screening for binding of the labelled LERK to the ERK. Alternatively, labelled NLERK2 may be used to screen expression libraries of putative ERK genes or functional parts thereof.

LERKs are important for the proliferation, differentiation and survival of a diverse array of cell types. Accordingly, it is proposed that NLERK2 or its functional derivatives be used to regulate development, maintenance or regeneration in an array of different cells and tissues *in vitro* and *in vivo*. For example, NLERK2 is contemplated to be useful in modulating neuronal proliferation, differentation and survival.

Soluble NLERK2 polypeptides are also contemplated to be useful in the treatment of disease, injury or abnormality in the nervous system, e.g. in relation to central or peripheral nervous system to treat Cerebral Palsy, trauma induced paralysis, vascular ischaemia associated with stroke, neuronal tumours, motoneurone disease, Parkinson's disease, Huntington's disease, Alzheimer's disease, Multiple Sclerosis, peripheral neuropathies associated with diabetes, heavy metal or alcohol toxicity, renal failure and infectious diseases such as herpes, rubella, measles, chicken pox, HIV or HTLV-1.

Membrane bound NLERK2 may be used *in vitro* on nerve cells or tissues to modulate proliferation, differentiation or survival, for example, in grafting procedures or transplantation.

As stated above, the NLERK2 of the present invention or its functional derivatives may be provided in a pharmaceutical composition comprising the LERK together with one or more pharmaceutically acceptable carriers and/or diluents. In addition, the present invention contemplates a method of treatment comprising the administration of an effective amount of a LERK of the present invention. The present invention also extends to antagonists and agonists of LERKs and their use in therapeutic compositions and methodologies.

A further aspect of the present invention contemplates the use of NLERK2 or its functional derivatives in the manufacture of a medicament for the treatment of NLERK2 mediated conditions defective or deficient.

The present invention is further described by the following non-limiting Figures and Examples.

In the Figures:

Figure 1 is a representation of the amino acid sequence of NLERK2 [SEQ ID NO: 6] of the present invention in comparison to known LERKs. LERK-1/B61 is described in International Patent Publication No. WO 95/06085; MCEK7/ELF1 in WO 96/10911 and NLERK1/ELF2 in WO 96/01839. The putative leader sequences, transmembrane sequences and GPI anchor sequences are shaded and boxed. Areas of amino acid sequence conservation are boxed, dashes signify gaps introduced to produce the alignment and potential N-glycosylation sites are underlined.

Figure 2 is a photographic representation showing expression of NLERK2. Panel A represents SDS-PAGE of extracellular medium from COS cells transfected with pEF sol NLERK2 cDNA (tracks a and b) or pEF fl NLERK2 cDNA (tracks c and d). Panel B represent SDS-PAGE of whole cell lysates from COS cells transfected with pEF sol NLERK2 cDNA (tracks a and b) or pEF fl NLERK2 cDNA (tracks c and d). (a, b are from two independent pEF sol NLERK2 clones and c, d are from two independent pEF fl NLERK2

clones). SDS-PAGE gels were transferred to PVDF filters and blotted with M2 anti-FLAG™ antibody (IBI/Kodak, CT, USA) followed by HRP-conjugated rabbit anti-mouse immunoglobulin (Dako code PO260, Denmark) and binding detected by enhanced chemiluminescence as described for Amersham ECL reagent (Amersham, Buckinghamshire, England).

Figure 3 is a schematic representation showing structure of NLERK2 cDNA and location of NLERK2 clones.

Figure 4 is a representation of the nucleotide sequence [SEQ ID NO:5] and corresponding amino acid sequence [SEQ ID NO:14] of full length NLERK2 cDNA. The leader sequence is lightly underlined, the transmembrane domain is heavily underlined and the potential N-glycosylation site is indicated by a bold N.

Figure 5 is a representation of the N-terminal amino acid sequence of pEF solNLERK2. The underlined portion is the C-terminal portion of the IL-3 signal sequence. The dotted underlined sequence is the sequence of the FLAG tag used to detect the expressed molecule. The double underlined sequence is the 5′ cloning site for the NLERK2 cDNA. The non-underlined residues are the mature 5′ coding region.

A summary of SEQ ID Nos used in this specification is given in Table 2.

TABLE 2

SUMMARY OF SEQ ID NOs

SEQUENCE	SEQ ID NO:
VXWXSXN	1
DXXDIXCP	2
EXYXLYXVXXXXXXXC	3
KFQXXXXXXXGXEFXXXHXYYXI	4
Nucleotide sequence of full length NLERK2	5
Amino acid sequence of NLERK2	6
Oligonucleotide	7
Oligonucleotide	8
Oligonucleotide	9
Oligonucleotide	10
Oligonucleotide	11
Oligonucleotide	12
Oligonucleotide	13
3' end of NLERK2 gene	14
5' end of NLERK2 gene	15
Signal sequence of murine IL-3	16
FLAG epitope	17

Single and triple letter abbreviations for amino acid residues are used in the subject specification, as defined in Table 3.

TABLE 3

AMINO ACID ABBREVIATIONS

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

EXAMPLE 1

RATIONALE

An approach was used based on conserved amino acid sequence elements in the LERKs to seek additional members of the family, reasoning that the large number of ERKs (at least 12) implied an equally large number of LERKs. The known LERK sequences were aligned and are shown in Figure 1. It was noted that there were small conserved amino acid sequence blocks, namely

(i) VXWXSXN [SEQ ID NO:1];

(ii) DXXDIXCP [SEQ ID NO:2];

(iii) EXYXLYXVXXXXXXXC [SEQ ID NO:3]; and/or

(iv) KFQXXXXXXXGXEFXXXHXYYXI [SEQ ID NO:4].

These amino acid sequence blocks were used to individually search the NCBI-Genbank data bank for any nucleotide sequences that, when translated in any of the six possible ways into amino acids (forward in each of three reading frames and backward in each of three reading frames), could encode a peptide corresponding to the above sequence blocks. For this purpose, the Pearson TFASTA search routine (Pearson WR and Lipman DJ *Proc. Natl. Acad. Sci. USA 85:* 2444-2448, 1988; and Pearson WR *Methods in Enzymology 183:* 63-98, 1990) was used. Using SEQ ID NO:4 resulted in highest scores for human LERK2 (Gene Bank Code No. HSU09304), rat ELK-L (Gene Bank Code No. RSU07560), human ELK-L (Gene Bank Code No. HUMEFL3), human EHK1-L (Gene Bank Code No. HUMEFL2), mouse LERK2 (Gene Bank Code No. MLERK2), human B61 (Gene Bank Code No. HUMB61), mouse ERF-1 (Gene Bank Code No. MMU14941), mouse cek 5-L (Gene Bank Code No. MMU12983).

The next highest score was for deposited Gene Bank Code No. 269886NCBI which represents a human-expressed sequence tag (EST) derived from a 73 day post-natal female whole brain cDNA library. Alignment and translation of an amino acid sequence predicted to be encoded by the EST (Gene Bank Code No. 269886NCBI) demonstrates it to be related to the known LERKs (Figure 1). The sequence does diverge, however, and hence encodes a novel human

LERK, referred to herein as "NLERK2" (Figures 1 and 4).

EXAMPLE 2

CLONING FULL LENGTH NLERK2 cDNA

Pooled oligos were used to probe library 53 which is a human fetal brain cDNA library. The oligos were synthesized according to the cDNA sequence of the expressed sequence tag [EST tag no. 269886NCBI]. The oligonucleotide probes comprise the following sequences:

(i)	5′	GGCAGGTTCTCCTTCCCCAGGCTCCCA 3′	[SEQ ID NO:7]
(ii)	5′	GTAGTAATCGTGGTGCGAGCG 3′	[SEQ ID NO:8]
(iii)	5′	GGCATGAAGGTGCTTCTCCGA 3′	[SEQ ID NO:9]

A number of duplicate positive hybridizing plaques were picked and purified on CsCl gradient and sequenced on both strands. Overlapping cDNA sequences from 5 independent clones were obtained encompassing the entire coding region and were sequenced on both strands (see Figure 4).

EXAMPLE 3

GENERATION OF EXPRESSION CONSTRUCTS

Constructs were generated to express full length and soluble forms of the protein. A derivative of the mammalian expression vector pEF-BOS (Mizushima *et al, Nucl. Acids. Res. 18:* 5322, 1990) was engineered to contain DNA encoding the signal sequence of murine IL-3 (MVLASSTTSIHTMLLLLLMLFHLGLQASIS [SEQ ID NO:16]) and the FLAG epitope (DYKDDDDK [SEQ ID NO:17]) followed by a unique Xba 1 cloning site. This vector was named pEF/IL3SIG/FLAG.

PCR was performed using oligos to amplify DNA fragments encoding the entire mature coding region from L28 to V340 using clones 53.1 and 53.8. The oligonucleotide probes comprise the following sequences:

(i) 5' AGCTTCTAGACTCAACCTGGAGCCTGTCTAC 3'
[SEQ ID NO:10]

(ii) 5' AGCTTCTAGATCATACCTTGTAGTAGATGTTTGG 3'
[SEQ ID NO:11]

PCR was used to amplify the extracellular domain without the transmembrane or cytoplasmic region. The sequences of the oligonucleotides used in this PCR are as follows:

(i) 5' AGCTTCTAGACTCAACCTGGAGCCTGTCTAC 3'
[SEQ ID NO:12]

(ii) 5' AGCTTCTAGATCAGCTGGGAGGGGGCAGGGGGCC 3'
[SEQ ID NO:13]

Full length and soluble forms were digested with XbaI. Both products were cloned in frame into the XbaI site of pEF/IL3SIG/FLAG to yield pEF fl NLERK2 and pEF sol NLERK2. The vectors are expressed in COS cells as described below.

In order to confirm that full-length and soluble forms of NLERK2 could be produced using the expression vectors pEF-flNLERK-2 and pEF-solNERK-2, COS cells were transiently transfected with these constructs. Briefly, COS cells from a confluent 175 cm^2 tissue culture flask were resuspended in PBS and electroporated (BioRad Gene pulser; 500 μF, 300 V) with 20 μg of uncut expression vector in a 0.4 cm cuvette (BioRad). After 2 to 3 days at 37°C in a fully humidified incubator containing 10% v/v CO_2 in air, cells were used for analyses of protein expression. Conditioned medium was collected by centrifugation, concentrated ten-fold, and stored sterile at 4°C. Cells were also harvested and lysed for 5 min in 500 μl of 50 mM Tris Hcl pH7.4 containing 150 mM NaCl, 2 mM EDTA and 1% v/v Triton X-100. The

intact nuclei were removed by centrifugation at 10,000g for 5 min. The cell lysate and conditioned medium were electrophoresed on pre-cast 12% w/v polyacrylamide gels (BioRad). The resolved proteins were then electroblotted onto PVDF membranes, which were then blocked with 5% skim milk, 0.1% v/v Tween 20 in PBS, rinsed and incubated with 5 μl of anti-FLAG M2 antibody (IBI) in 2.5 ml of PBS containing 5% skim milk and 0.1% v/v Tween 20. The membrane was then rinksed and incubated with peroxidase-conjugated human anti-mouse Ig in 5% skim milk, 0.1% v/v Tween in PBS, rinsed and incubated with ECL reagent (Amersham, Buckinghamshire, UK) or 1 min. Filters were then blotted dry and exposed to autoradiographic film for 1 min.

Figure 2 shows expression of NLERK2 in COS cells. The apparent molecular mass of soluble and full length NLERK2 were 32 and 50 kDa, respectively, suggesting glycosylation.

Figure 5 shows the N-terminal amino acid sequence of anti-FLAG M2 antibody purified soluble NLERK2 from pEF solNLERK2. This protein is transiently expressed in COS cells (Figure 2). Single underlined sequence is the C-terminal portion of the IL-3 signal sequence which was cleaved before the Alanine. The dotted underlined sequence is sequence of the FLAG tag used to detect the expressed molecule. The double underlined sequence is the 5' cloning site for the NLERK2 cDNA. The non-underlined residues are the mature 5' coding region (lacking the leader sequence). The italicised Asparagine (*N*) was modified for cloning reasons from the Serine residue of the sequence given in Figure 4.

The full length nucleotide sequence [SEQ ID NO:5] and corresponding amino acid sequence [SEQ ID NO:6] is shown in Figure 4.

EXAMPLE 4

FLANKING REGIONS OF NLERK2 GENE

The 5′ and 3′ portions of the NLERK2 genomic gene were determined and are shown in SEQ ID NO:15 and SEQ ID NO:14, respectively.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT (countries other than US): AMRAD OPERATIONS PTY. LTD.
(US only): NICOS ANTHONY NICOLA

(ii) TITLE OF INVENTION: NOVEL RECEPTOR LIGANDS AND GENETIC SEQUENCES ENCODING SAME

(iii) NUMBER OF SEQUENCES: 17

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 19-JUL-1996

(vii) PRIOR APPLICATION DATA:
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(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Val Xaa Trp Xaa Ser Xaa Asn
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Xaa Xaa Asp Ile Xaa Cys Pro
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Xaa Tyr Xaa Leu Tyr Xaa Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Lys Phe Gln Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Xaa Glu Phe Xaa Xaa
1               5                  10                  15

Xaa His Xaa Tyr Tyr Xaa Xaa Ile
            20
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1021 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG GGC CCC CCC CAT TCT GGG CCG GGG GGC GTG CGA GTC GGG GCC CTC      48
Met Gly Pro Pro His Ser Gly Pro Gly Gly Val Arg Val Gly Ala Leu
 1               5                  10                  15

CTC CTA CTG GGG GTT TTG GGG CTG GTG TCT GGG CTC AGC CTG GAG CCT      96
Leu Leu Leu Gly Val Leu Gly Leu Val Ser Gly Leu Ser Leu Glu Pro
            20                  25                  30

GTC TAC TGG AAC TCG GCG AAT AAG AGG TTC CAG GCA GAG GGT GGT TAT     144
Val Tyr Trp Asn Ser Ala Asn Lys Arg Phe Gln Ala Glu Gly Gly Tyr
        35                  40                  45
```

```
GTG CTG TAC CCT CAG ATC GGG GAC CGG CTA GAC CTA CTC TGC CCC CGG      192
Val Leu Tyr Pro Gln Ile Gly Asp Arg Leu Asp Leu Leu Cys Pro Arg
     50                  55                  60

GCC CGG CCT CCT GGC CCT CAC TCC TCT CCT AAT TAT GAG TTC TAC AAG      240
Ala Arg Pro Pro Gly Pro His Ser Ser Pro Asn Tyr Glu Phe Tyr Lys
 65                  70                  75                  80

CTG TAC CTG GTA GGG GGT GCT CAG GGC CGA CGC TGT GAG GCA CCC CCT      288
Leu Tyr Leu Val Gly Gly Ala Gln Gly Arg Arg Cys Glu Ala Pro Pro
                85                  90                  95

GCC CCA AAC CTC CTT CTC ACT TGT GAT CGC CCA GAC CTG GAT CTC CGC      336
Ala Pro Asn Leu Leu Leu Thr Cys Asp Arg Pro Asp Leu Asp Leu Arg
            100                 105                 110

TTC ACC ATC AAG TTC CAG GAG TAT AGC CCT AAT CTC TGG GGC CAC GAG      384
Phe Thr Ile Lys Phe Gln Glu Tyr Ser Pro Asn Leu Trp Gly His Glu
        115                 120                 125

TTC CGC TCG CAC CAC GAT TAC TAC ATC ATT GCC ACA TCG GAT GGG ACC      432
Phe Arg Ser His His Asp Tyr Tyr Ile Ile Ala Thr Ser Asp Gly Thr
    130                 135                 140

CGG GAG GGC CTG GAG AGC CTA CAG GGA GGT GTG TGC CTA ACC AGA GGC      480
Arg Glu Gly Leu Glu Ser Leu Gln Gly Gly Val Cys Leu Thr Arg Gly
145                 150                 155                 160

ATG AAG GTG CTT CTC CGA GTG GGA CAA AGT CCC CGA GGA GGG GCT GTC      528
Met Lys Val Leu Leu Arg Val Gly Gln Ser Pro Arg Gly Gly Ala Val
                165                 170                 175

CCC CGA AAA CCT GTG TCT GAA ATG CCC ATG GAA AGA GAC CGA GGG GCA      576
Pro Arg Lys Pro Val Ser Glu Met Pro Met Glu Arg Asp Arg Gly Ala
            180                 185                 190

GCC CAC AGC CTG GAG CCT GGG AAG GAG AAC CTG CCA GGT GAC CCC ACC      624
Ala His Ser Leu Glu Pro Gly Lys Glu Asn Leu Pro Gly Asp Pro Thr
        195                 200                 205

AGC AAT GCA ACC TCC CGG GGT GCT GAA GGC CCC CTG CCC CCT CCC AGC      672
Ser Asn Ala Thr Ser Arg Gly Ala Glu Gly Pro Leu Pro Pro Pro Ser
    210                 215                 220
```

```
ATG CCT GCA GTG GCT GGG GCA GCA GGG GGG CTG GCG CTG CTC TTG CTG      720
Met Pro Ala Val Ala Gly Ala Ala Gly Gly Leu Ala Leu Leu Leu Leu
225                 230                 235                 240

GGC GTG GCA GGG GCT GGG GGT GCC ATG TGT TGG CGG AGA CGG CGG GCC      768
Gly Val Ala Gly Ala Gly Gly Ala Met Cys Trp Arg Arg Arg Arg Ala
                245                 250                 255

AAG CCT TCG GAG AGT CGC CAC CCT GGT CCT GGC TCC TTC GGG AGG GGA      816
Lys Pro Ser Glu Ser Arg His Pro Gly Pro Gly Ser Phe Gly Arg Gly
            260                 265                 270

GGG TCT CTG GGC CTG GGG GGT GGA GGT GGG ATG GGA CCT CGG GAG GCT      864
Gly Ser Leu Gly Leu Gly Gly Gly Gly Gly Met Gly Pro Arg Glu Ala
        275                 280                 285

GAG CCT GGG GAG CTA GGG ATA GCT CTG CGG GGT GGC GGG GCT GCA GAT      912
Glu Pro Gly Glu Leu Gly Ile Ala Leu Arg Gly Gly Gly Ala Ala Asp
    290                 295                 300

CCC CCC TTC TGC CCC CAC TAT GAG AAG GTG AGT GGT GAC TAT GGG CAT      960
Pro Pro Phe Cys Pro His Tyr Glu Lys Val Ser Gly Asp Tyr Gly His
305                 310                 315                 320

CCT GTG TAT ATC GTG CAG GAT GGG CCC CCC CAG AGC CCT CCA AAC ATC     1008
Pro Val Tyr Ile Val Gln Asp Gly Pro Pro Gln Ser Pro Pro Asn Ile
                325                 330                 335

TAC TAC AAG GTA TGA
Tyr Tyr Lys Val  *
            340
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 340 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Gly Pro Pro His Ser Gly Pro Gly Gly Val Arg Val Gly Ala Leu
 1               5                  10                  15
```

Leu Leu Leu Gly Val Leu Gly Leu Val Ser Gly Leu Ser Leu Glu Pro
20 25 30

Val Tyr Trp Asn Ser Ala Asn Lys Arg Phe Gln Ala Glu Gly Gly Tyr
35 40 45

Val Leu Tyr Pro Gln Ile Gly Asp Arg Leu Asp Leu Leu Cys Pro Arg
50 55 60

Ala Arg Pro Pro Gly Pro His Ser Ser Pro Asn Tyr Glu Phe Tyr Lys
65 70 75 80

Leu Tyr Leu Val Gly Gly Ala Gln Gly Arg Arg Cys Glu Ala Pro Pro
85 90 95

Ala Pro Asn Leu Leu Leu Thr Cys Asp Arg Pro Asp Leu Asp Leu Arg
100 105 110

Phe Thr Ile Lys Phe Gln Glu Tyr Ser Pro Asn Leu Trp Gly His Glu
115 120 125

Phe Arg Ser His His Asp Tyr Tyr Ile Ile Ala Thr Ser Asp Gly Thr
130 135 140

Arg Glu Gly Leu Glu Ser Leu Gln Gly Gly Val Cys Leu Thr Arg Gly
145 150 155 160

Met Lys Val Leu Leu Arg Val Gly Gln Ser Pro Arg Gly Gly Ala Val
165 170 175

Pro Arg Lys Pro Val Ser Glu Met Pro Met Glu Arg Asp Arg Gly Ala
180 185 190

Ala His Ser Leu Glu Pro Gly Lys Glu Asn Leu Pro Gly Asp Pro Thr
195 200 205

Ser Asn Ala Thr Ser Arg Gly Ala Glu Gly Pro Leu Pro Pro Pro Ser
210 215 220

Met Pro Ala Val Ala Gly Ala Ala Gly Gly Leu Ala Leu Leu Leu Leu
225 230 235 240

Gly Val Ala Gly Ala Gly Gly Ala Met Cys Trp Arg Arg Arg Arg Ala
245 250 255

```
Lys Pro Ser Glu Ser Arg His Pro Gly Pro Gly Ser Phe Gly Arg Gly
            260                 265                 270

Gly Ser Leu Gly Leu Gly Gly Gly Gly Gly Met Gly Pro Arg Glu Ala
        275                 280                 285

Glu Pro Gly Glu Leu Gly Ile Ala Leu Arg Gly Gly Gly Ala Ala Asp
    290                 295                 300

Pro Pro Phe Cys Pro His Tyr Glu Lys Val Ser Gly Asp Tyr Gly His
305                 310                 315                 320

Pro Val Tyr Ile Val Gln Asp Gly Pro Pro Gln Ser Pro Pro Asn Ile
                325                 330                 335

Tyr Tyr Lys Val  *
            340
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCAGGTTCT CCTTCCCCAG GCTCCCA 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTAGTAATCG TGGTGCGAGC G 21

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGCATGAAGG TGCTTCTCCG A 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGCTTCTAGA CTCAACCTGG AGCCTGTCTA C 31

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCTTCTAGA TCATACCTTG TAGTAGATGT TTGG 34

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGCTTCTAGA CTCAACCTGG AGCCTGTCTA C 31

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGCTTCTAGA TCAGCTGGGA GGGGGCAGGG GGCC 34

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1692 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGAGGGCTCC TCTCACGTGG CTATCCTGAA TCCAGCCCTT 40

CCTGGGGTGC TCCTCCAGTT TAATTCCTGG TTTGAGGGAC ACCTCTAACA TCTCGGCCCC 100

CTGTGCCCCC CCAGCCCCTT CACTCCTCCC GGGTGCTGTC CTCGTCTCCA CTTTTAGGAT 160

TCCTTAGGAT TCCCACTGCC CCACTTCCTG CCCTCCCGTT TGGCCATGGG TGCCCCCCTC 220

TGTCTCAGTG TCCCTGGATC CTTTTTCCTT GGGGAGGGGC ACAGGCTCAG CCTCCTCTCT 280

GACCATGAMC CAGGGATCCT TGTCCCCCTC AMCCACCCAG AGCTAGGGGG CGGGAACAGC 340

CCACCTTTTG GTTGGCACCG CCTTCTTTCT GCCTCTCACT GGTTTTCTCT TCTCTATCTC 400

TTATTCTTTC CCTCTCTTCC GTCTCTAGGT CTGTTCTTCT TCCCTAGCAT CCTCCTCCCC 460

ACATCTCCTT TCACCCTCTT GGCTTCTTAT CCTGTGCCTC TCCCATCTCC TGGGTGGGGS 520

SATCAAAGCA TTTCTCCCCT TAGCTTTCAS CCCCCCTTCT GACCTCTCAT ACCAACCACT 580

CCCCTCAGTC TSYCAAAAAT GGGGGCCTTA TGGGGAAGGC TCTGACACTC CACCCCAGCT 640

CAGGCCATGG GCAGCAGGGC TCCATTCTCT GGCCTGGCCC AGGCCTCTAC ATACTTACTC 700

CAGCCATTTG GGGTGGTTGG GTCATGACAG CTACCATGAG AAGAAGTGTC CCGTTTTGTC 760

CAGTGGCCAA TAGCAAGATA TGAACCGGTC GGGACATGTA TGGACTTGGT CTGATGCTGA 820

ATGGGCCACT TGGGACCGGA AGTGACTTGC TCCAGACAAG AGGTGACCAG GCCCGGACAG 880

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GCTGATTTTA GGACAGCTAA GATGACTGCC ATGTGCTGTG GCAGGCCTAA TTTGTCTTGT 1240

TCTTTCCTTT CCATATCCCA GTATAATCTC TGTTAATCAA CAGGACTACC CCAAGAACCC 1300

ATGTGCTCTC CCGAGTAACC CAGATGGCTG TCTTGTTCAT TCCATCCTAC ATTTCTGACT 1360

CCTTTCAGAC TCAACACAGT TCCCTTCTTA GTGACCACAA TGGTGGCCTA CTGCCTGGTC 1420

TAGCTGACAG TGGTACTTAG CAMAGGCCAC TGTTTCCATA GTGACCAGCT GATACCTCTT 1480

CCTGCCCTCT AGTGTGCAAT TGGGTGTTGC CTCAGTTTCC TCCCAGCTCA GTTTTATTAG 1540

ATCAAACCTG TTGTTGGGCA CCAGGTTGTC CACCTCAATC ACCAGCCAAG ATGGTTGCTT 1600

TGTCCACCAG AGGTCAAGTT CACCTCTCTG GTGCTGTAGT TCCCAGCTCC TTCCTGATTT 1660

TTCTAATGGA ATTCGATATC AAGCTTATCG AT 1692

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 399 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTGGAGCCCA CGCGGTGGCG GTCGCTCTAG AATAGTGGAT CCCCCGGGCT GCAGGAATTC 60

CAGCCCCTTC CTCGCTCCCT GGTCCGGCGC CCCATGCCGC CCCCGCCCGG TCCCCGGYTC 120

CCCCAGTCCC CCACTTAGGC GGGATCACAG ATCCCGGGGT GCTGGCGCGT GGGCCGGGGG 180

CGCGTAGGGC GCCTGCAGAC GGCCCCTGGA AGGGCTCTGG TGGGGCTGAG CGCTCTGCCG 240

CGGGGGCGCG GGCACAGCAG GAAGCAGGTC CGCGTGGGCG CTGGGGGCAT CAGCTACCGG 300

GGTGGTCCGG GCTGAAGAGC CAGGCASCAA GGCAGCCACC CCGGGGGGTG GGCGACTTTG 360

GGGGAGTTGG TGCGCCCCCC CCCAGACCTT GACGGGGTC 399

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Val Leu Ala Ser Ser Thr Thr Ser Ile His Thr Met Leu Leu Leu
5 10 15

Leu Leu Met Leu Phe His Leu Gly Leu Gln Ala Ser Ile Ser
20 25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Tyr Lys Asp Asp Asp Asp Lys
5

CLAIMS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a LERK or part thereof, said nucleotide sequence or its complementary form capable of encoding at least one of the following amino acid sequences:

(i) VXWXSXN [SEQ ID NO:1];

(ii) DXXDIXCP [SEQ ID NO:2];

(iii) EXYXLYXVXXXXXXXC [SEQ ID NO:3]; and/or

(iv) KFQXXXXXXXGXEFXXXHXYYXI [SEQ ID NO:4],

wherein X is any amino acid residue.

2. An isolated nucleic acid molecule according to claim 1 encoding at least two of SEQ ID NO:1 to SEQ ID NO:4.

3. An isolated nucleic acid molecule according to claim 1 encoding at least three of SEQ ID NO:1 to SEQ ID NO:4.

4. An isolated nucleic acid molecule according to claim 1 selected from the group consisting of:

(i) a nucleic acid molecule having a nucleotide sequence substantially as set forth in SEQ ID NO:5;

(ii) a nucleic acid molecule having a nucleotide sequence having at least 75% similarity to the sequence set forth in SEQ ID NO:5; and

(iii) a nucleic acid molecule capable of hybridising under low stringency conditions to the nucleotide sequence or its complementary form set forth in SEQ ID NO:5.

5. An isolated nucleic acid molecule according to claim 4 further comprising a sequence of nucleotides as set forth in SEQ ID NO:14 and/or SEQ ID NO:15 or having at least 75%

similarity thereto and/or is capable of hybridising thereto under low stringency conditions.

6. An isolated nucleic acid molecule according to claim 4 or 5 obtainable from the human genome.

7. An isolated nucleic acid molecule according to claim 4 or 5 wherein the human genome is in brain, liver, kidney, neonatal, embryonic, cancer or tumour-derived tissue.

8. A method for cloning a nucleotide sequence encoding a novel LERK said method comprising searching a nucleotide database for a sequence which encodes at least one of amino acid sequences SEQ ID NO:1 to SEQ ID NO:4, designing one or more oligonucleotide primers based on a nucleotide sequence located in the search, screening a nucleic acid library with said one or more oligonucleotides and obtaining a clone therefrom which encodes said novel LERK.

9. A method according to claim 8 wherein the nucleic acid library is a cDNA expression library.

10. A method according to claim 9 wherein the cDNA expression library is of human origin derived from brain, liver, kidney, neonatal, embryonic, tumour or cancer tissue.

11. A method according to claim 8 wherein the oligonucleotide primers are selected from SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13.

12. A method according to claim 8 wherein the cloned nucleotide sequence is selected from the listing consisting of:

(i) a nucleotide sequence substantially as set forth in SEQ ID NO:5;

(ii) a nucleotide sequence having at least 75% similarity to the sequence set forth in SEQ ID NO:5; and

(iii) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence or its complementary form set forth in SEQ ID NO:5.

13. A recombinant polypeptide having the ability to interact with an ERK wherein said recombinant polypeptide comprises at least one of amino acid sequence SEQ ID NO:1 to SEQ ID NO:4.

14. A recombinant polypeptide according to claim 13 having an amino acid sequence substantially as set forth in SEQ ID NO:6 or a functional part thereof.

15. A pharmaceutical composition comprising a recombinant polypeptide as defined in claim 12 and one or more pharmaceutically acceptable carriers and/or diluents.

16. A pharmaceutical composition according to claim 15 wherein the polypeptide comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or a functional part thereof.

17. A use of a recombinant polypeptide having an amino acid sequence substantially as set forth in SEQ ID NO:6 or a functional part thereof in the manufacture of a medicament for the treatment of disease conditions resulting from NLERK2-deficiency or NLERK2-defectiveness.

18. A nucleic acid comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:14 or having at least 75% similarity thereto or is capable of hybridising thereto under low stringency conditions.

19. A nucleic acid comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:15 or having at least 75% similarity thereto or is capable of hybridising thereto under low stringency conditions.

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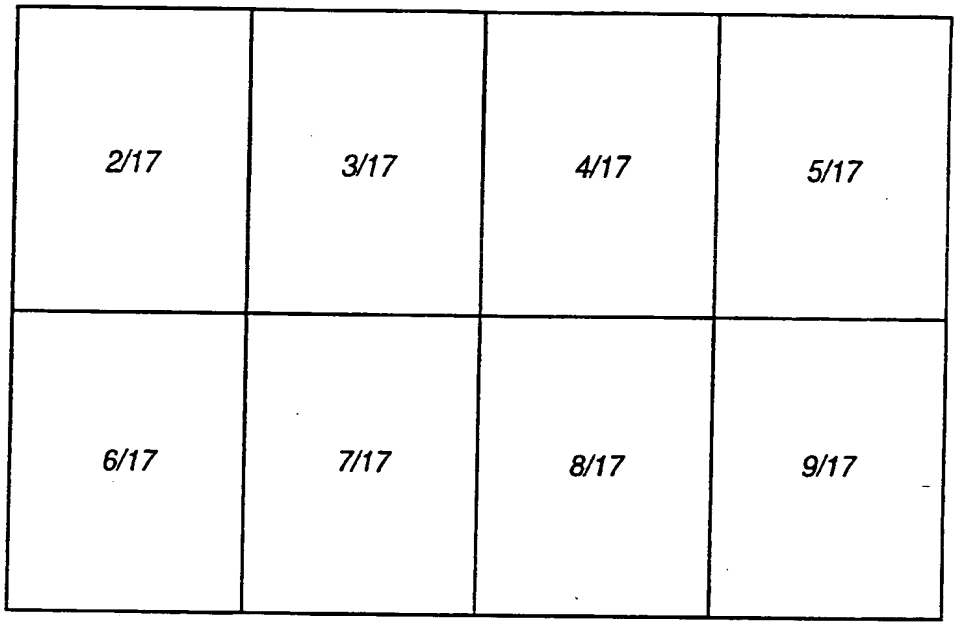

Fig.1

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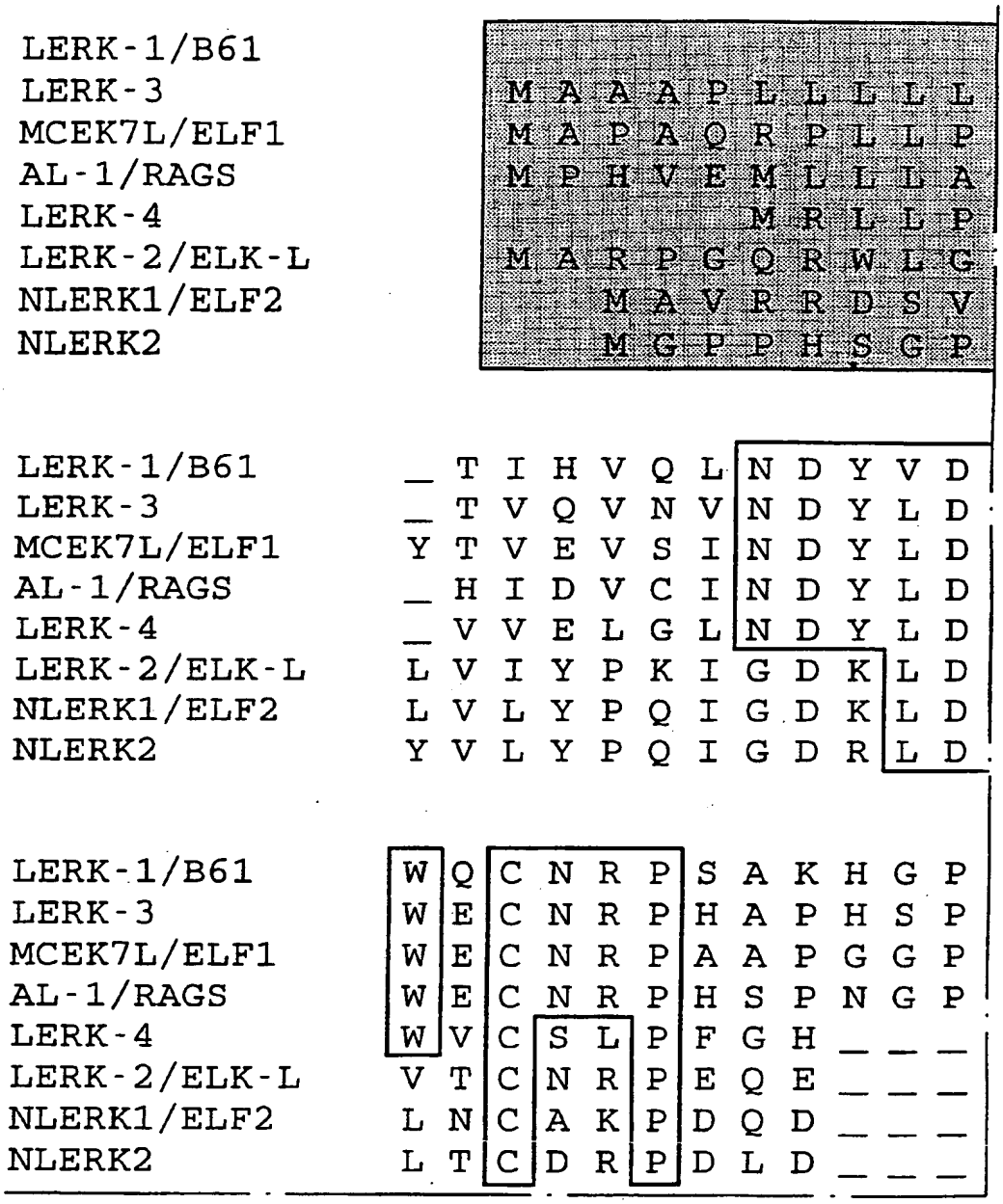
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MCEK7L/ELF1 M P H V E M L L L A
AL-1/RAGS M R L L P
LERK-4
LERK-2/ELK-L M A R P G Q R W L G
NLERK1/ELF2 M A V R R D S V
NLERK2 M G P P H S G P
LERK-1/B61 _ T I H V Q L N D Y V D
LERK-3 _ T V Q V N V N D Y L D
MCEK7L/ELF1 Y T V E V S I N D Y L D
AL-1/RAGS _ H I D V C I N D Y L D
LERK-4 _ V V E L G L N D Y L D
LERK-2/ELK-L L V I Y P K I G D K L D
NLERK1/ELF2 L V L Y P Q I G D K L D
NLERK2 Y V L Y P Q I G D R L D
LERK-1/B61 W Q C N R P S A K H G P
LERK-3 W E C N R P H A P H S P
MCEK7L/ELF1 W E C N R P A A P G G P
AL-1/RAGS W E C N R P H S P N G P
LERK-4 W V C S L P F G H _ _ _
LERK-2/ELK-L V T C N R P E Q E _ _ _
NLERK1/ELF2 L N C A K P D Q D _ _ _
NLERK2 L T C D R P D L D _ _ _

Fig.1

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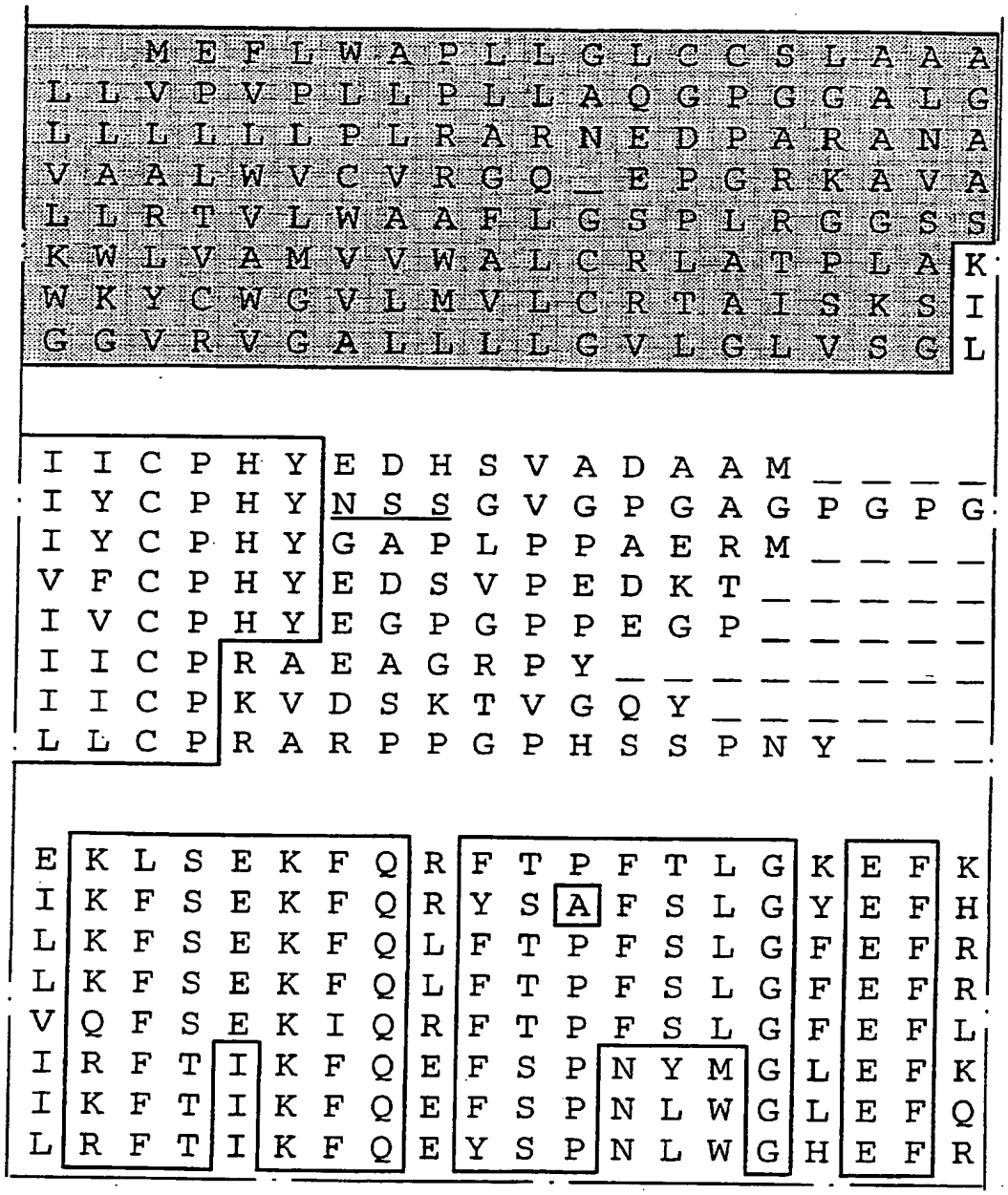

Fig.1

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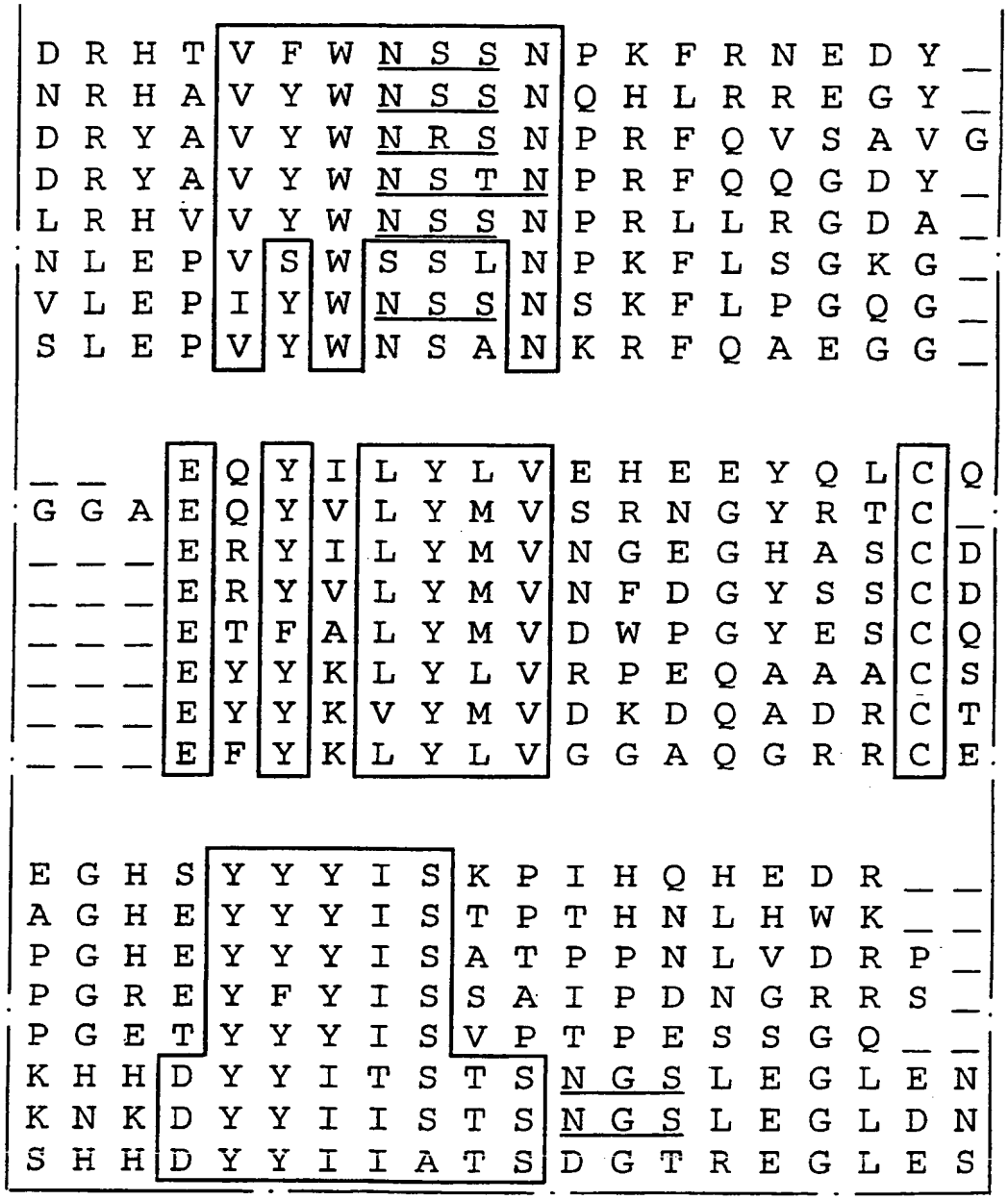
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N L E P V S W S S L N P K F L S G K G _
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_ _ E Q Y I L Y L V E H E E Y Q L C Q
G G A E Q Y V L Y M V S R N G Y R T C _
_ _ _ E R Y I L Y M V N G E G H A S C D
_ _ _ E R Y V L Y M V N F D G Y S S C D
_ _ _ E T F A L Y M V D W P G Y E S C Q
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Fig.1

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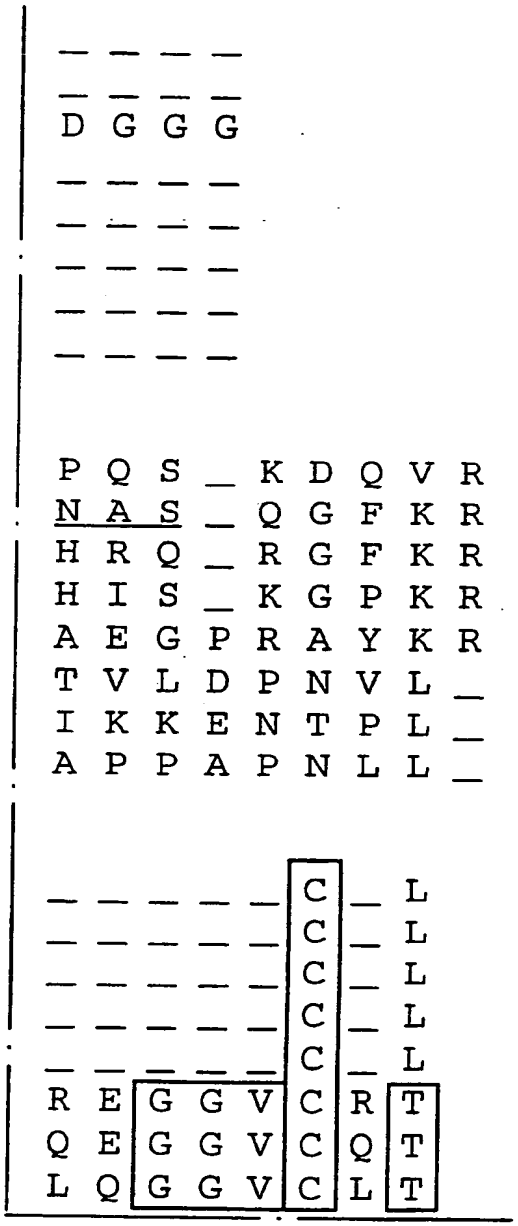
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H R Q _ R G F K R
H I S _ K G P K R
A E G P R A Y K R
T V L D P N V L _
I K K E N T P L _
A P P A P N L L _
R E G G V C R T
Q E G G V C Q T
L Q G G V C L T

Fig.1

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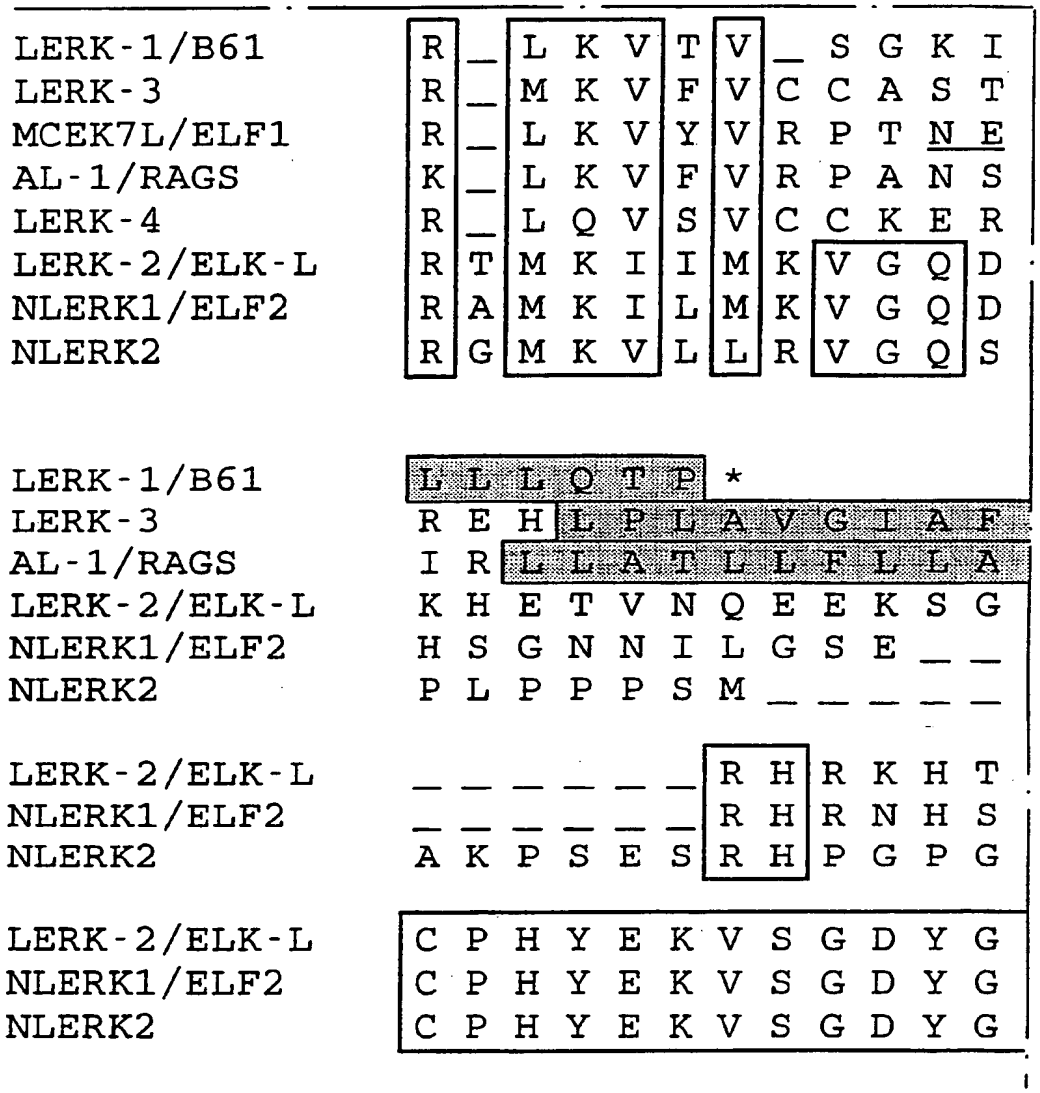
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LERK-3 R _ M K V F V C C A S T
MCEK7L/ELF1 R _ L K V Y V R P T N E
AL-1/RAGS K _ L K V F V R P A N S
LERK-4 R _ L Q V S V C C K E R
LERK-2/ELK-L R T M K I I M K V G Q D
NLERK1/ELF2 R A M K I L M K V G Q D
NLERK2 R G M K V L L R V G Q S
LERK-1/B61 L L L Q T P *
LERK-3 R E H L P L A V G I A F
AL-1/RAGS I R L L A T L L F L L A
LERK-2/ELK-L K H E T V N Q E E K S G
NLERK1/ELF2 H S G N N I L G S E _ _
NLERK2 P L P P P S M _ _ _ _ _
LERK-2/ELK-L _ _ _ _ _ _ R H R K H T
NLERK1/ELF2 _ _ _ _ _ _ R H R N H S
NLERK2 A K P S E S R H P G P G
LERK-2/ELK-L C P H Y E K V S G D Y G
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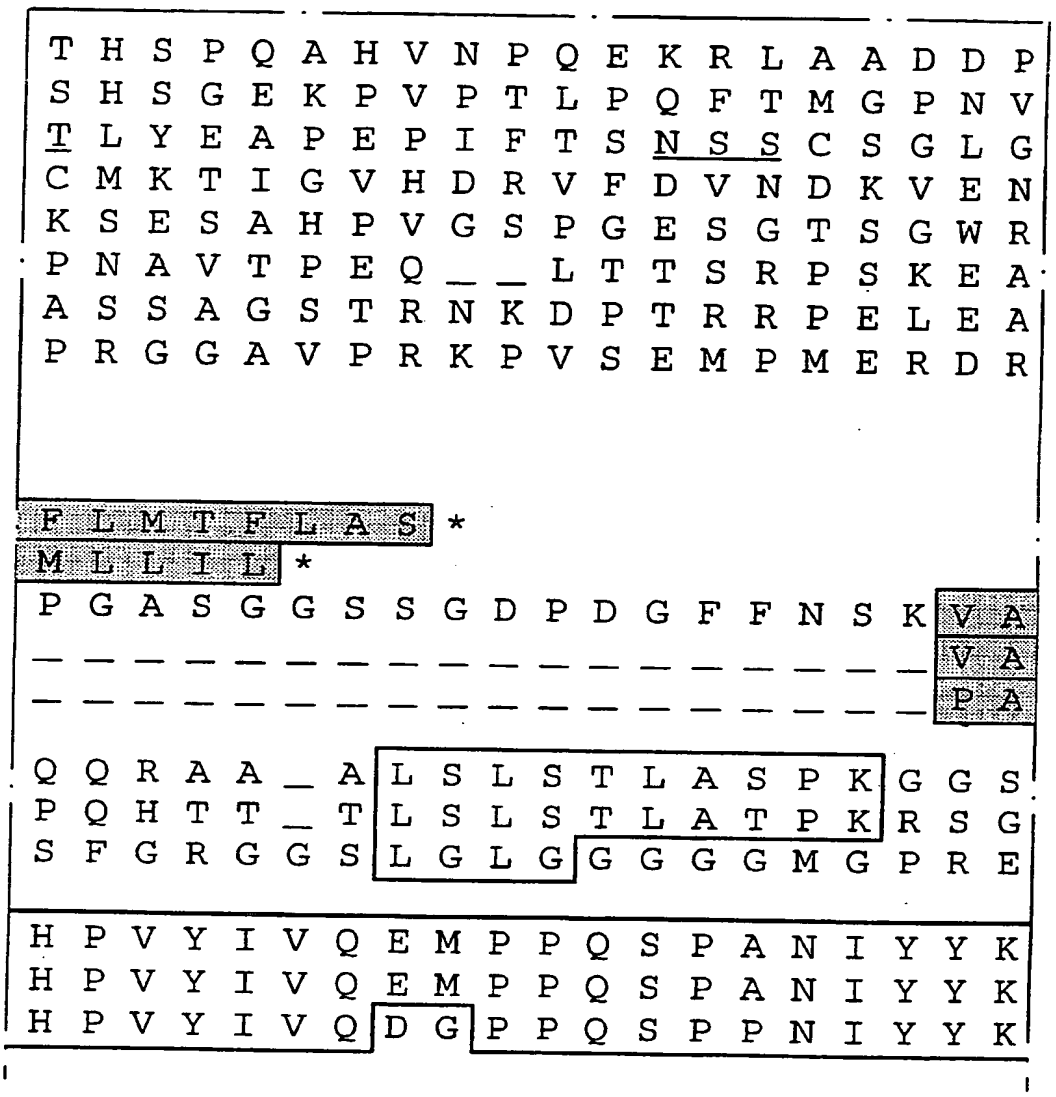
NLERK2 C P H Y E K V S G D Y G

Fig. 1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T L Y E A P E P I F T S N S S C S G L G
C M K T I G V H D R V F D V N D K V E N
K S E S A H P V G S P G E S G T S G W R
P N A V T P E Q _ _ L T T S R P S K E A
A S S A G S T R N K D P T R R P E L E A
P R G G A V P R K P V S E M P M E R D R
F L M T F L A S *
M L L I L *
P G A S G G S S G D P D G F F N S K V A
V A
P A
Q Q R A A _ A L S L S T L A S P K G G S
P Q H T T _ T L S L S T L A T P K R S G
S F G R G G S L G L G G G G G M G P R E
H P V Y I V Q E M P P Q S P A N I Y Y K
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H P V Y I V Q D G P P Q S P P N I Y Y K

Fig. 1

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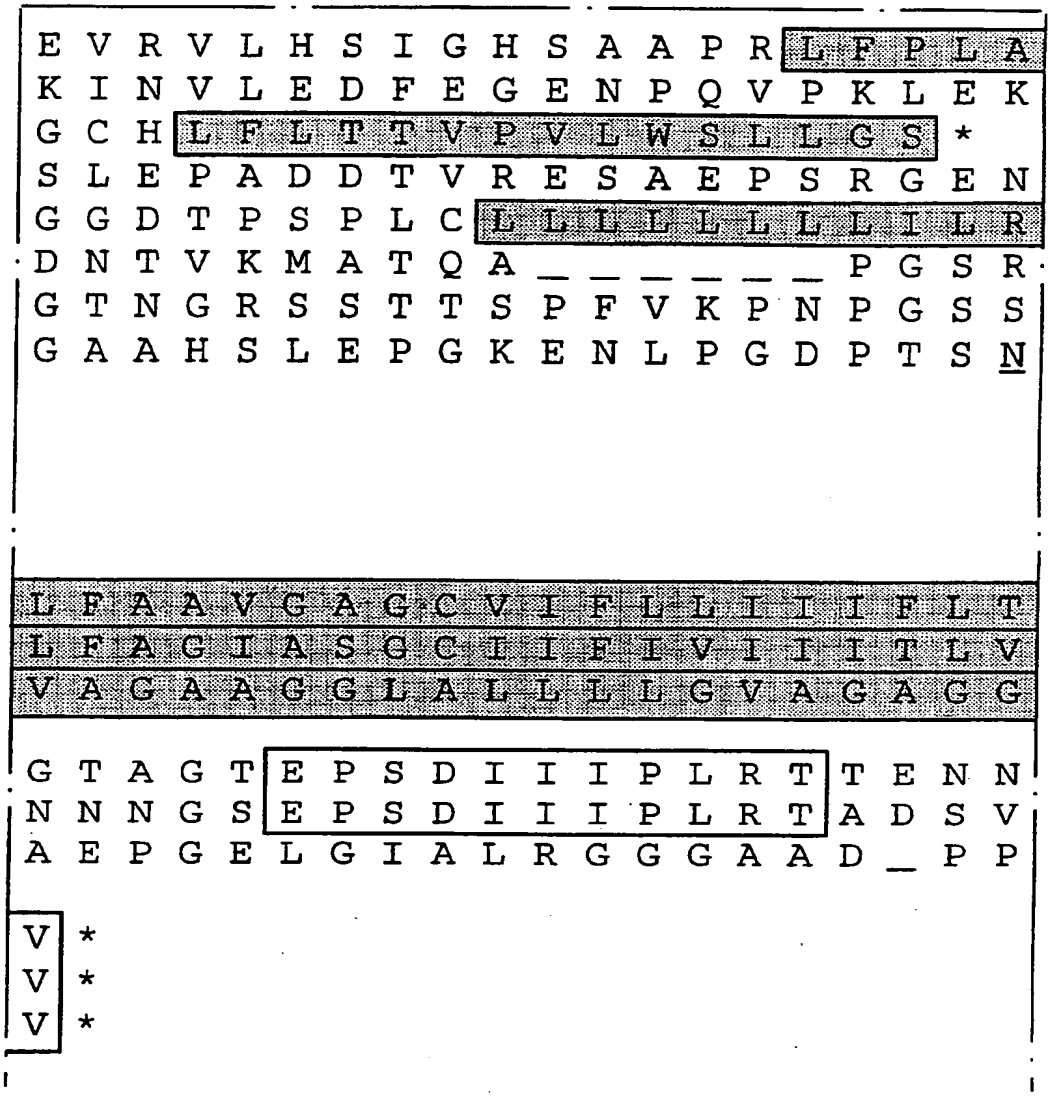
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G C H L F L T T V P V L W S L L G S *
S L E P A D D T V R E S A E P S R G E N
G G D T P S P L C L L L L L L L L I L R
D N T V K M A T Q A _ _ _ _ _ _ P G S R
G T N G R S S T T S P F V K P N P G S S
G A A H S L E P G K E N L P G D P T S N
L F A A V G A G C V I F L L I I I F L T
L F A G I A S G C I I F I V I I I T L V
V A G A A G G L A L L L L G V A G A G G
G T A G T E P S D I I I P L R T T E N N
N N N G S E P S D I I I P L R T A D S V
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V *
V *
V *

Fig. 1

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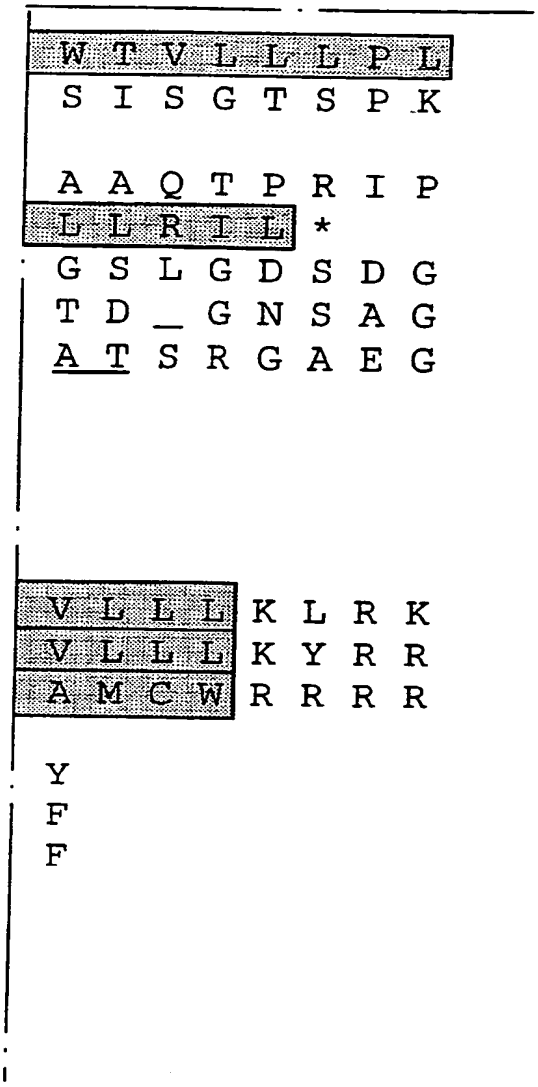
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L L R I L *
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A M C W R R R R
Y
F
F

Fig. 1

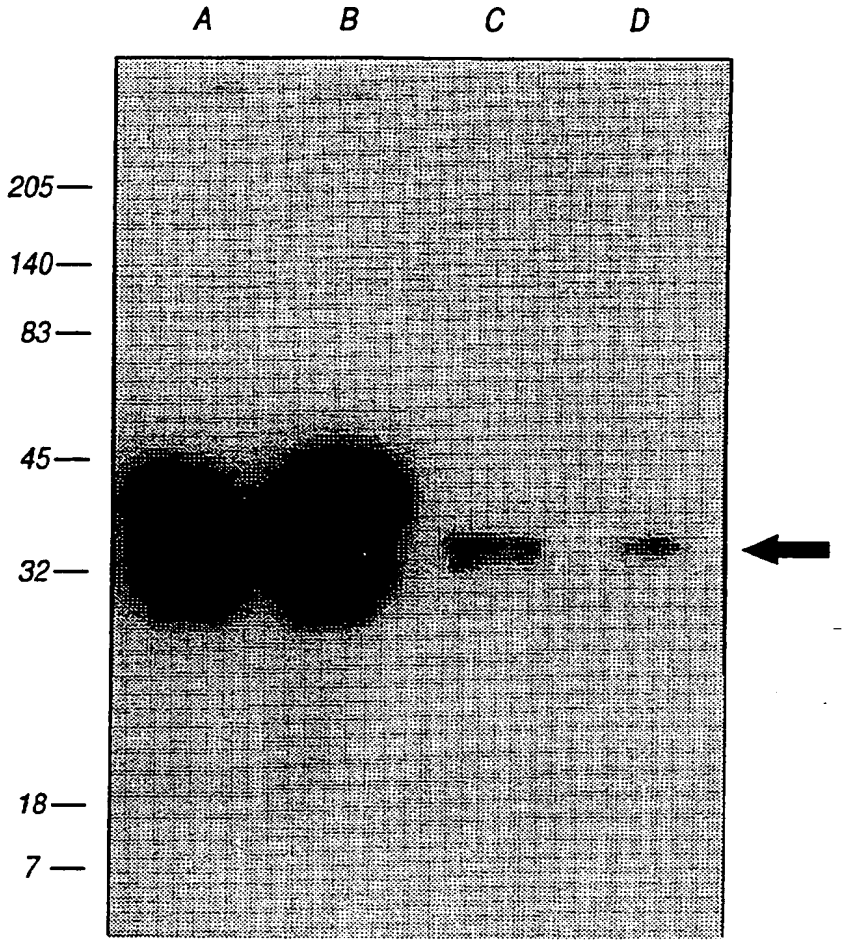

Fig. 2A

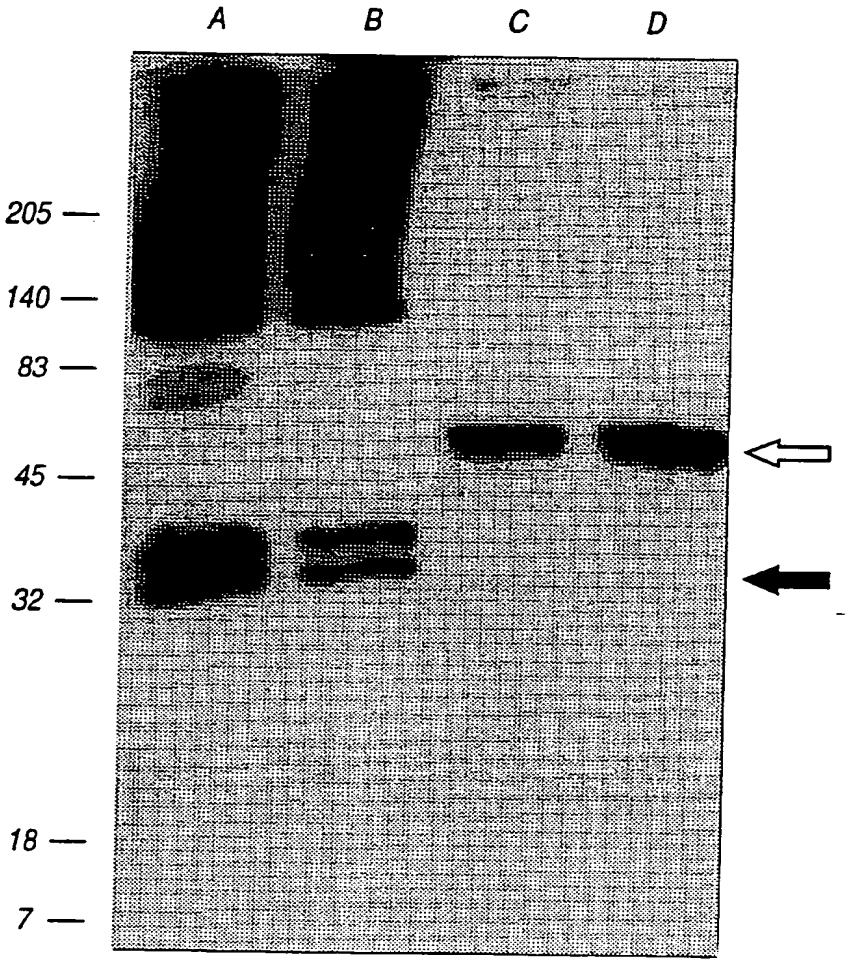

Fig. 2B

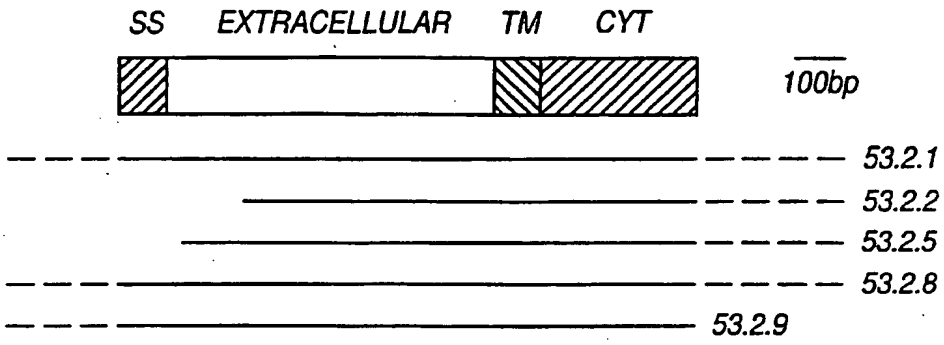

Fig.3

ASISSSDYKDDDESRLNLEPVYW

Fig.5

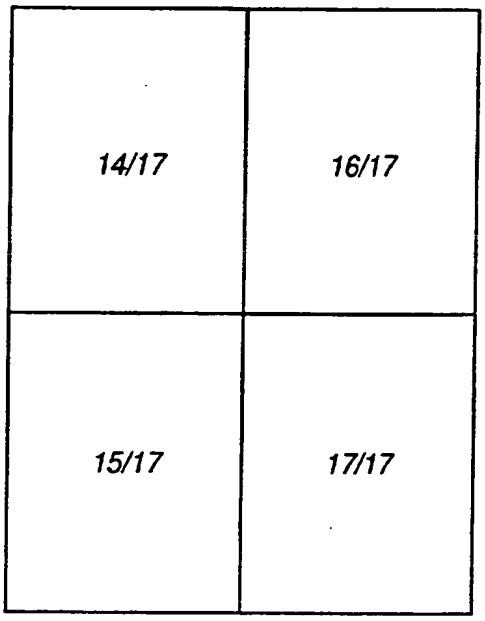

Fig.4

```
1     ATGGGCCCCCCCCATTCTGGGCCGGGGGGC
1      M  G  P  P  H  S  G  P  G  G

61    GTTTTGGGGCTGGTGTCTGGGCTCAGCCTG
21     V  L  G  L  V  S  G  L  S  L

121   AGGTTCCAGGCAGAGGGTGGTTATGTGCTG
41     R  F  Q  A  E  G  G  Y  V  L

181   CTCTGCCCCCGGGCCCGGCCTCCTGGCCCT
61     L  C  P  R  A  R  P  P  G  P

241   CTGTACCTGGTAGGGGGTGCTCAGGGCCGA
81     L  Y  L  V  G  G  A  Q  G  R

301   CTTCTCACTTGTGATCGCCCAGACCTGGAT
101    L  L  T  C  D  R  P  D  L  D

361   AGCCCTAATCTCTGGGGCCACGAGTTCCGC
121    S  P  N  L  W  G  H  E  F  R

421   TCGGATGGGACCCGGGAGGGCCTGGAGAGC
141    S  D  G  T  R  E  G  L  E  S

481   ATGAAGGTGCTTCTCCGAGTGGGACAAAGT
161    M  K  V  L  L  R  V  G  Q  S
```

Fig. 4

```
541  GTGTCTGAAATGCCCATGGAAAGAGACCGA
181    V  S  E  M  P  M  E  R  D  R

601  GAGAACCTGCCAGGTGACCCCACCAGCAAT
201    E  N  L  P  G  D  P  T  S  N

661  CCCCCTCCCAGCATGCCTGCAGTGGCTGGG
221    P  P  P  S  M  P  A  V  A  G

721  GGCGTGGCAGGGGCTGGGGGTGCCATGTGT
241    G  V  A  G  A  G  G  A  M  C

781  AGTCGCCACCCTGGTCCTGGCTCCTTCGGG
261    S  R  H  P  G  P  G  S  F  G

841  GGTGGGATGGGACCTCGGGAGGCTGAGCCT
281    G  G  M  G  P  R  E  A  E  P

901  GGGGCTGCAGATCCCCCCTTCTGCCCCCAC
301    G  A  A  D  P  P  F  C  P  H

961  CCTGTGTATATCGTGCAGGATGGGCCCCCC
321    P  V  Y  I  V  Q  D  G  P  P

1021 tga
       *
```

Fig. 4

```
GTGCGAGTCGGGGCCCTCCTCCTACTGGGG
 V  R  V  G  A  L  L  L  L  G

GAGCCTGTCTACTGGAACTCGGCGAATAAG
 E  P  V  Y  W  N  S  A  N  K

TACCCTCAGATCGGGGACCGGCTAGACCTA
 Y  P  Q  I  G  D  R  L  D  L

CACTCCTCTCCTAATTATGAGTTCTACAAG
 H  S  S  P  N  Y  E  F  Y  K

CGCTGTGAGGCACCCCCTGCCCCAAACCTC
 R  C  E  A  P  P  A  P  N  L

CTCCGCTTCACCATCAAGTTCCAGGAGTAT
 L  R  F  T  I  K  F  Q  E  Y

TCGCACCACGATTACTACATCATTGCCACA
 S  H  H  D  Y  Y  I  I  A  T

CTACAGGGAGGTGTGTGCCTAACCAGAGGC
 L  Q  G  G  V  C  L  T  R  G

CCCCGAGGAGGGGCTGTCCCCCGAAAACCT
 P  R  G  G  A  V  P  R  K  P
```

Fig. 4

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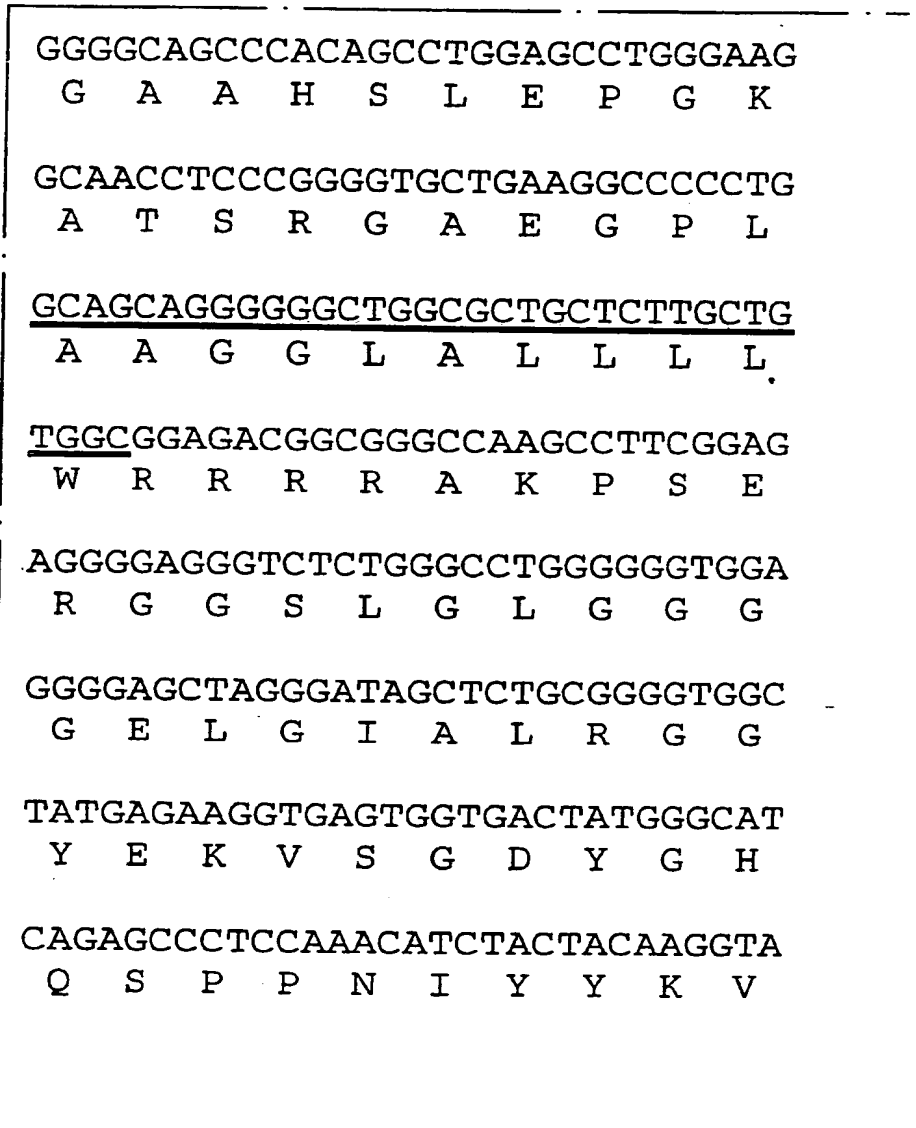
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A A G G L A L L L L
TGGCGGAGACGGCGGGCCAAGCCTTCGGAG
W R R R R A K P S E
AGGGGAGGGTCTCTGGGCCTGGGGGGTGGA
R G G S L G L G G G
GGGGAGCTAGGGATAGCTCTGCGGGGTGGC
G E L G I A L R G G
TATGAGAAGGTGAGTGGTGACTATGGGCAT
Y E K V S G D Y G H
CAGAGCCCTCCAAACATCTACTACAAGGTA
Q S P P N I Y Y K V

Fig. 4

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00460

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/12; C07K 2/00, 4/12, 14/435; A61K 38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC6: Chemical Abstracts: keywords below.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Medline, JAPIO: keywords below.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, JAPIO
MEDLINE, CA: keywords: Lerk or RPTK; tyrosine kinase receptor or EPH or ERK or EHK or ECK or EFL.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Oncogene, Volume 10 (2) issued January 1995, Kozlosky et al, "Ligands for the receptor tyrosine kinases hek and ehk: isolation of cDNA's encoding a family of proteins" pages 299-306. See entire document.	1-19
X	Cell, Volume 79, issued October 1994, Cheng and Flanagan "Identification of ELF-1 a developmentally expressed ligand for the Mek 4 and Sek receptor tyrosine kinases" pages 157-168. See entire document.	1-19
X	Science, volume 266, issued November 1994, Davies et al "Ligands for EPH - Related receptor tyrine kinases that require membrane attachment or clustering for activity" pages 816-819. See entire document.	1-19

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 1996	02.10.96
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	Authorized officer KAREN AYERS Telephone No.: (06) 283 2082

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00460

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	WO 96/10911 (Immunex Corp.) 18 April 1996, see entire document in particular example 1 and SEQ ID NO. 2	1-19
PX	WO 96/17925 (Immunex Corp) 13 June 1996, see entire document, in particular example 1 and SEQ ID No. 5.	1-19

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 96/00460

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	9617925	AU	46393/96
WO	9610911	AU	38268/95

END OF ANNEX

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